Biodistribution and shedding analysis following treatment with RP1 oncolytic immunotherapy in the skin cancer patients from the IGNYTE clinical trial: Implications for pharmacy and other clinical staff

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Background

- RP1 is a genetically modified herpes simplex virus type 1 (HSV-1)–based oncolytic immunotherapy (OI) that selectively replicates in and kills tumors[1-2]
- RP1 is under evaluation in a phase 1/2 open-label, multicenter, dose-escalation and dose-expansion trial evaluating the safety and efficacy of RP1 in combination with the anti–PD-1 antibody nivolumab in a range of tumor types (NCT03767348)[3]
- RP1 is delivered intratumorally via injection into superficial lesions or deeper tumors using image guidance. Example handling of HSV-1–based OIs is shown in **Figure 1**. As the field of OIs continues to grow, the importance of understanding biosafety considerations is essential for pharmacy and nursing staff

Figure 1. Example handling of HSV-1 oncolytic immunotherapies[4]

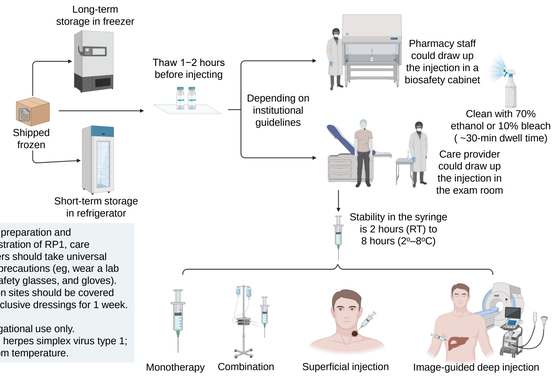


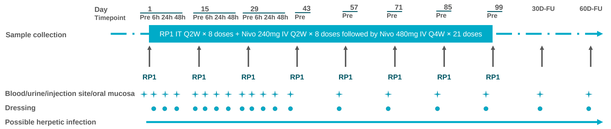
Figure adapted from: Robilotti E, et al. *Front Mol BioSci*. 2023. doi 10.3389/fmolb.2023.117832. © 2023 Robilotti, Zeitouni and Orloff.

Objective

To assess the biodistribution and shedding patterns of RP1 from the patients enrolled in the cutaneous melanoma and anti–PD-1 naïve non-melanoma skin cancer (NMSC) cohorts (n = 61) in the IGNYTE trial.

Sample collection schema

Figure 2: Sample type and collection schedule

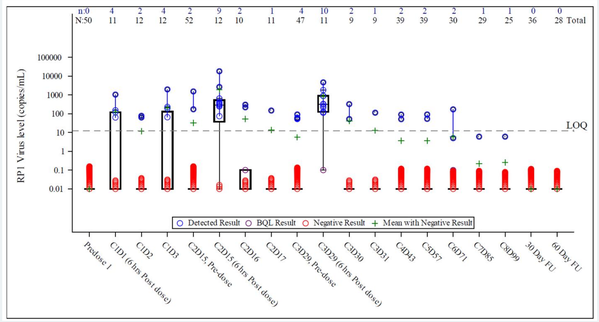


FU, follow-up; IT, intratumoral; IV, intravenous; Q2W, every 2 weeks; Q4W, every 4 weeks.

Blood, urine, and swabs from the exterior of occlusive dressings, the surface of injection sites, the oral mucosa, and any areas of suspected herpetic infection origin were collected throughout the study (**Figure 2**). The presence of RP1 DNA was assessed using an RP1-specific and sensitive qPCR assay. qPCR-positive swab samples were further tested for infectious virus in validated 50% tissue culture infective dose (TCID50) assay.

Results

Figure 3. RP1 DNA levels in blood

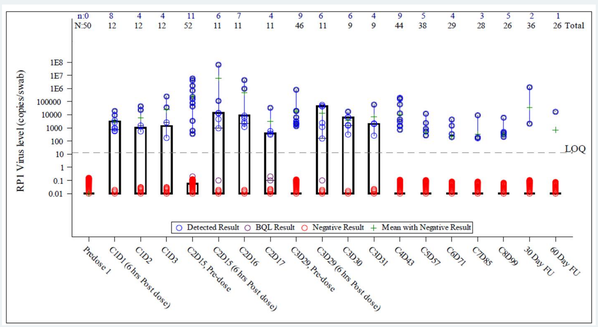


BQL, below quantitation limit; C#D#, cycle number, study day number; FU, follow-up; hrs, hours; N, number of patients tested; n, number of patients with DNA virus level equal to or above the lower limit of quantification; LOQ, limit of quantification

Blood: The highest levels of RP1 DNA copy numbers were detectable in blood shortly (6 hrs) after injection. A subset of patients showed continued presence of RP1 DNA throughout to the next injection, 15 days later, suggesting RP1 replication in tumors (**Figure 3**).

Urine: Throughout the 8 cycles, RP1 DNA was undetectable in urine samples: 0/53 patients and 0/453 samples (**Tables 1 and 2**).

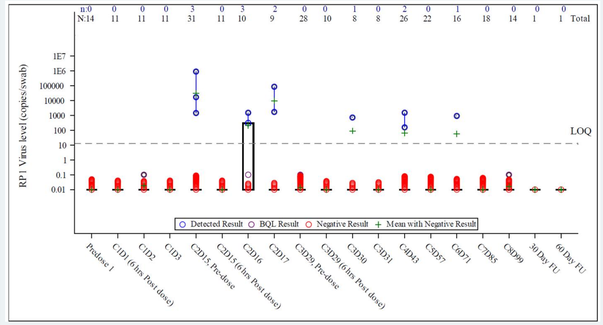
Figure 4. RP1 DNA levels at the site of injection



BQL, below quantitation limit; C#D#, cycle number, study day number; FU, follow-up; hrs, hours; N, number of patients tested; n, number of patients with DNA Virus Level equal to or above the lower limit of quantification; LOQ, limit of quantification

Injection site: The incidence of RP1 DNA was highest during Cycle 2 with approximately 20% of patients having detectable levels at the injection site after 15 days post-RP1 injection (**Figure 4**). During the safety follow-up period, RP1 DNA was only detected on the surface of injected lesions and not at any other sites (eg, blood, urine).

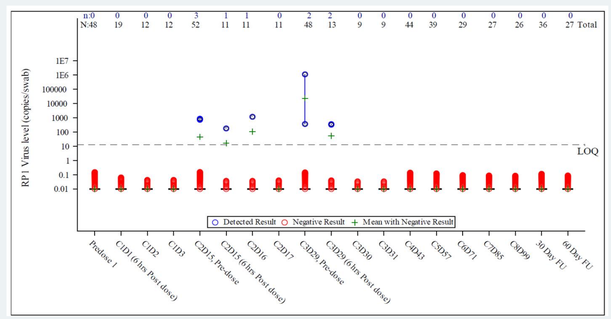
Figure 5. RP1 DNA levels from the exterior dressing



BQL, below quantitation limit; C#D#, cycle number, study day number; FU, follow-up; hrs, hours; N, number of patients tested; n, number of patients with DNA Virus Level equal to or above the lower limit of quantification; LOQ, limit of quantification

Exterior dressings: RP1 DNA copies detected from dressings were lower compared with the number of copies detected at the site of injection (**Tables 1 and 2; Figure 5**). RP1 DNA remained undetectable from all dressing samples collected post Cycle 6 Day 71.

Figure 6. RP1 DNA levels from oral mucosa/saliva



BQL, below quantitation limit; C#D#, cycle number, study day number; FU, follow-up; hrs, hours; N, number of patients tested; n, number of patients with DNA Virus Level equal to or above the lower limit of quantification; LOQ, limit of quantification

Oral mucosa: RP1 DNA was rarely detected and only at low levels on oral mucosa (**Figure 6**), with 8/53 (15.1%) patients and 9/483 (1.9%) samples testing positive (**Tables 1 and 2**).

No live virus was detected from the swab samples that tested positive for RP1 DNA: All swab samples (107 from the surface of injection site, 16 from exterior dressings, 9 from oral mucosa) which tested positive for RP1 DNA were assessed for the presence of infectious virus by the TCID50 assay, and all tested negative for infectivity. There were no reported infections of staff or caregivers.

Patient and sample incidence of RP1 DNA detection

Table 1: Patient incidence of RP1 DNA detection[a]

	HSV-1 seronegative	HSV-1 seropositive	Overall
Blood	8/11 (72.7)	9/42 (21.4)	17/53 (32.1)
Urine	0/11 (0)	0/42 (0)	0/53 (0)
Mucosa	1/11 (9.1)	7/42 (16.7)	8/53 (15.1)
Injection site	10/11 (90.9)	17/42 (40.5)	27/53 (50.9)
Dressing exterior	3/11 (27.3)	5/28 (17.9)	8/39 (20.5)

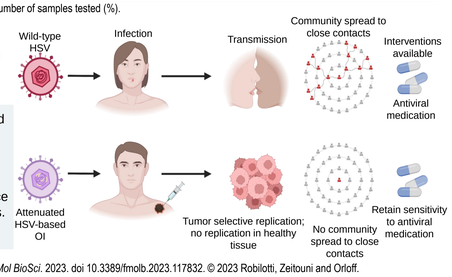
[a]Data indicate number of patients positive/number of patients tested (%).

Table 2: Sample incidence of RP1 DNA detection[a]

	HSV-1 seronegative	HSV-1 seropositive	Overall
Blood	34/140 (24.3)	18/332 (5.4)	52/472 (11.0)
Urine	0/135 (0)	0/318 (0)	0/453 (0)
Mucosa	1/138 (0.7)	8/345 (2.3)	9/483 (1.9)
Injection site	48/138 (34.8)	59/334 (17.7)	107/472 (22.7)
Dressing exterior	10/101 (9.9)	6/157 (3.8)	16/258 (6.2)

[a]Data indicate number of samples positive/number of samples tested (%).

Figure 7. Differences between wild-type HSV and HSV-based OIs[4]



Wild-type HSV may replicate and transmit to others, potentially resulting in community spread. Attenuated HSV-based OIs are limited to replication within tumors and do not show evidence of transmission to close contacts.
HSV, herpes simplex virus; OI, oncolytic immunotherapy.

Figure adapted from: Robilotti E, et al. *Front Mol BioSci*. 2023. doi 10.3389/fmolb.2023.117832. © 2023 Robilotti, Zeitouni and Orloff.

Conclusions

- No infectious RP1 virus was detected from any swab sample; only residual RP1 DNA was concluded to be present
- RP1 DNA was detected primarily in injection-site samples
- RP1 DNA was found less frequently on exterior dressing samples, suggesting that the dressings act as an effective barrier
- Overall, RP1 showed negligible potential for viral transmission to pharmacy staff and other caregivers, patients, and their families, with no evidence of transmission having been reported
- As pharmacy staff and other caregivers incorporate the use of viral oncolytic immunotherapies into patient care, biodistribution and shedding data will be important to evaluate during development of internal protocols for handling of these agents

The IGNYTE trial is now recruiting patients. To learn more about enrolling your patient, contact: clinicaltrials@replimune.com or +1 (781) 222 9570.

Additional information can be obtained by visiting Clinicaltrials.gov (NCT03767348)

References:

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2. Chmielowski B, et al. *J Clin Oncol.* 2023;41(16_suppl):9509-9509.
3. Middleton M, et al. *J Clin Oncol.* 2020;38(15):e22050.
4. Robilotti E, et al. *Front Mol BioSci*. 2023. doi 10.3389/fmolb.2023.117832.

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